FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
220631US0PCT	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
10/070899	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/JP00/06226	12 September 2000
PRIORITY DATE CLAIMED	
13 September 1999 (earliest)	

TITLE OF INVENTION
NITROIMIDAZOLE DERIVATIVE AND DIAGNOSTIC IMAGING AGENT CONTAINING THE SAME

APPLICANT(S) FOR DO/EO/US
TAKAI Yoshihiro et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include itens (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

PCT/IB/304
PCT/IB/308
Form PTO-1449
Request for Priority

Rec'd PCT/PTO 13 MAR 2002

U.S. APPLICATION NO. (IF KNOWN, SEE [illegible])	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/070899	PCT/JP00/06226	220631US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	14 - 20 =	0	x $18.00	**$0.00**	
Independent claims	2 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☒	**$280.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,300.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,300.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,300.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,300.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,300.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

22850

SIGNATURE

Norman F. Oblon William E. Beaumont

NAME

24,618 Registration Number 30,996

REGISTRATION NUMBER

March 13, 2002

DATE

APPLICATION DATA SHEET

APPLICATION INFORMATION

Application Number::	10/070,899
Application Date::	03/13/02
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CD-ROM or CD-R?::	NONE
Title::	NITROIMIDAZOLE DERIVATIVE AND DIAGNOSTIC IMAGING AGENT CONTAINING THE SAME
Attorney Docket Number::	220631US0PCT

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CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 22850

REPRESENTATIVE INFORMATION

Representative Customer Number:: 22850

DOMESTIC PRIORITY INFORMATION

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	National Stage of	PCT/JP00/06226	09/12/00

FOREIGN PRIORITY INFORMATION

Application Number:	Country::	Filing Date::	Priority Claimed::
11/259057	Japan	09/13/99	YES
11/260315	Japan	09/14/99	YES

ASSIGNMENT INFORMATION

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Postal or Zip Code of Mailing Address::	989-3214

Description

Nitroimidazole Derivative and Diagnostic Imaging Agent Containing the same

Technical Field

The present invention relates to a novel nitroimidazole derivative which is useful as a diagnostic imaging agent.

Background Art

In recent years, everyday foods in Japan have been Europeanized or Americanized, and in accordance with this trend, patients suffering diseases of the circulatory system, such as hyperlipidemia, angina pectoris, and myocardial infarction, have been drastically increasing. Such a disease may cause damage to nutrition-supplying organs of the body, such as the heart and blood vessels, and may be life threatening, depending on the progress of the disease. Therefore, the site of the disease must be determined at early stages of the disease and the disease must be subjected to appropriate treatment.

In ischemic diseases, peripheral tissues of ischemic sites are destroyed by active oxygen, and thus it is important not only to find the presence of vasoconstriction sites or heart valve disorder, but also to determine ischemic sites which are generated due to lack of blood flow. Briefly, damaged tissues at such ischemic sites, as well as

vasoconstriction sites and cardiac dysfunction, may be life threatening.

In recent years, diseases of circulatory organs have been reliably diagnosed, and the sites of the diseases have been precisely determined through angiography, electrocardiogram, load electrocardiogram, or 24-hour monitoring. However, even when such a method is employed, ischemic sites or tissues cannot be detected directly, and biopsy has been the main means for detecting damage derived from ischemia. Therefore, there has been demand for means to determine ischemic sites conveniently and reliably.

Meanwhile, in treatment of cancer, it is important to detect the presence of cancer cells at early stages of tumor formation, in order to enhance the effect of chemotherapy or radiotherapy or to arrest the progress of cancer, such as by preventing metastasis. In recent years, it has been reported that, among cancer cells, there are hypoxic cells which are resistant to a chemotherapeutic agent or radiation. Therefore, the amount of such hypoxic cells and the position at which the cells exist must be detected, and then the cells must be eliminated.

Conventionally, a method in which a monoclonal antibody against a tumor marker is employed is known as a typical method for detecting and identifying cancer cells. However, in the method, although the presence or the amount of a tumor maker is determined, the position at which the marker is present cannot be detected.

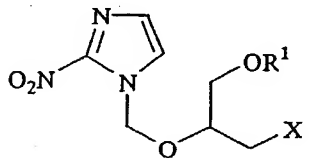
In order to detect the position at which a tumor is present, there has been carried out an imaging method, such as magnetic resonance imaging (MRI) in which the distribution of water is determined through proton NMR, or X-ray imaging by use of an organic iodine compound. However, such a method is carried out for merely detecting difference in biophysical properties of the tumor, which is attributed to the cancer tissue, and the method does not image cancer cells directly. Therefore, such a method does not provide information about the presence of chemotherapeutic-agent-resistant or radiation-resistant cells, the information being an index of difficulty in treatment of the tumor.

In order to solve the aforementioned problems, an object of the present invention is to provide a compound which is useful for imaging cancer cells or ischemic sites of circulatory organs.

Disclosure of the Invention

In view of the foregoing, the present inventors have performed extensive studies, and have found that a nitroimidazole derivative represented by the following formula (1) is selectively directed to ischemic sites of circulatory organs, or chemotherapeutic-agent-resistant or radiation-resistant hypoxic cancer cells; and that when the derivative is employed as a contrast medium in diagnostic imaging, the cells can be imaged. The present invention has been accomplished on the basis of these findings.

Accordingly, the present invention provides a nitroimidazole derivative represented by the following formula (1):

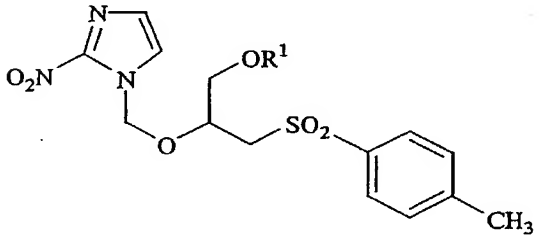


(1)

[wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group, and X represents a fluorine atom or an isotope thereof].

The present invention also provides a diagnostic imaging agent comprising the nitroimidazole derivative (1) as an active ingredient.

The present invention also provides a nitroimidazole derivative represented by the following formula (2):



(2)

[wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group], and a method for producing the nitroimidazole derivative represented by formula (1), comprising fluorination of the nitroimidazole derivative of formula (2).

Brief Description of Drawings

Fig. 1 is an autoradiogram of an ischemic heart, which is obtained by use of the diagnostic imaging agent of the present invention.

Fig. 2 is an autoradiogram of a tumor, which is obtained by use of the diagnostic imaging agent of the present invention.

Best Mode for Carrying Out the Invention

A nitroimidazole derivative represented by formula (1) of the present invention is a novel compound, and a fluorine atom or an isotope thereof represented by X in the formula is a stable isotope of fluorine (^{19}F) or a radioisotope of fluorine (^{18}F). When X is the radioisotope (^{18}F), the position of the derivative of the present invention in the body can be visualized through positron emission tomography (PET). When X is the non-radioactive stable isotope (^{19}F), the position of the derivative in the body can be visualized through MRI or a similar means. The derivative which does not comprise the radioisotope of fluorine plays an important role in imaging as an agent diluting the derivative comprising the radioisotope.

A C1-C4 alkanoyl group represented by R^1 may be an acetyl group, a propionyl group, a butyryl group, or an isobutyryl group, and is preferably an acetyl group.

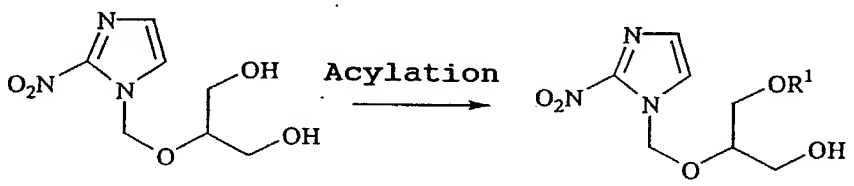
In the present invention, R^1 is more preferably a hydrogen atom, in consideration of control of imaging.

In the present invention, examples of preferable

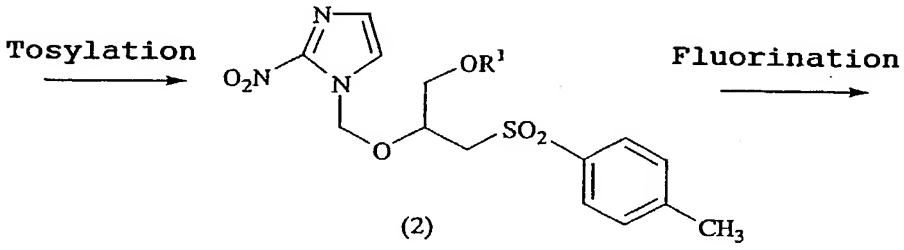
nitroimidazole derivatives (1) include 1-[2-fluoro(^{18}F or ^{19}F)-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole and 1-[1-acetoxymethyl-2-fluoro(^{18}F or ^{19}F)ethoxy]methyl-2-nitroimidazole.

The compound of the present invention (1) contains an asymmetric carbon atom, and thus there exist stereoisomers of the compound which are derived from the position of the carbon atom. The present invention encompasses the stereoisomers, and the streoisomers may be employed singly or in combination.

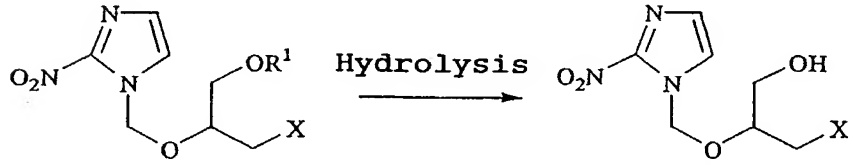
The nitroimidazole derivative (1) of the present invention may be produced through, for example, the following steps:



(A) (B)



(2)



(1-A) (1-B)

[wherein R^1 and X are identical to the aforementioned R^1 and X].

Firstly, a hydroxy form (A) is acylated to produce an ester form (B), and then the ester form is tosylated to produce a tosyl form (2) serving as a novel intermediate. Subsequently, the tosyl form is fluorinated, producing a nitroimidazole derivative (1-A) of the present invention in which R^1 is an alkanoyl group. If desired, the derivative (1-A) may be subjected to hydrolysis, to thereby obtain a nitroimidazole derivative (1-B) of the present invention, in which R^1 is hydrogen.

Acylation may be carried out through a customary method; for example, may be carried out by use of an acid halide in a solvent at -30 to 100°C for one to five hours in the presence or absence of an inorganic base, an organic base, or an organometallic compound. In acylation, an inorganic base may be potassium hydroxide, sodium carbonate, or cesium carbonate; an organic base may be pyridine, 4-dimethylaminopyridine, picoline, N,N-dimethylaniline, N-methylmorpholine, dimethylamine, triethylamine, or 1,8-diazabicyclo[5.4.0]undecene (DBU); and an organometallic compound may be dibutyl tin oxide. Examples of solvents which may be employed include halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, and chlorobenzene; aromatic hydrocarbons such as benzene and toluene; ethers such as tetrahydrofuran, diethyl ether, and dioxane; ketones such as acetone and methyl ethyl ketone;

non-protonic polar solvents such as acetonitrile and N,N-dimethylformamide; and ethyl acetate.

Tosylation may be carried out through a customary method; for example, may be carried out by use of 2-3 mol of tosyl halide (e.g., tosyl chloride) with respect to 1 mol of a material compound in the presence of a base such as triethylamine in an organic solvent such as methylene chloride, acetonitrile, dimethylformamide, or pyridine at 0-100°C for one to five hours.

Fluorination may be carried out in an inert solvent by use of crown ether serving as a catalyst and by use of a fluorination agent such as an alkali metal fluoride (e.g., sodium fluoride, potassium fluoride, or cesium fluoride) or a tetraammonium fluoride (e.g., tetrabutylammonium fluoride). An inert solvent is preferably a halogen solvent, an ether solvent, a hydrocarbon solvent, a polar solvent, or a solvent mixture thereof. Fluorination is usually carried out at about 70-130°C, and preferably at 100-120°C in the case in which DMF is employed as a solvent.

When a fluoride of ^{18}F (e.g., $K^{18}F$) is employed as a fluorination agent, fluorination is preferably carried out by use of cryptofix 2.2.2 serving as a phase transfer catalyst. A source of fluoride of ^{18}F can be obtained by trapping an aqueous solution of ^{18}F with an anion exchange resin and eluting the solution with an aqueous solution of potassium carbonate, the ^{18}F solution being obtained from enriched $H_2{}^{18}O$ by means of ^{18}O (p, n).

Hydrolysis may be carried out in the presence of an inorganic base in a solvent at 0-100°C for one to five minutes. An inorganic base may be potassium hydroxide, sodium hydroxide, potassium carbonate, sodium carbonate, or cesium carbonate. A solvent may be water; an alcohol such as methanol, ethanol, or propanol; an ether such as tetrahydrofuran, diethyl ether, or dioxane; or a ketone such as acetone or methyl ethyl ketone.

When the thus-produced nitroimidazole derivative (1) of the present invention is administered to a living organism, as shown in the below-described Test Examples, the derivative recognizes ischemic sites or a cancer cells and is rapidly directed thereto. Therefore, the derivative is useful as a diagnostic imaging agent, and when it is employed together with an apparatus for diagnostic imaging such as MRI, the position at which ischemic sites or cancer cells exist can be detected and the amount of the sites or cells can be measured.

The nitroimidazole derivative (1) of the present invention may be mixed with a pharmaceutically acceptable additive, to thereby produce a diagnostic imaging agent. Examples of such additives include pharmaceutically acceptable isotonic agents, emulsifying and dispersing agents, excipients, binders, coating agents, stabilizers, sugars such as mannitol, and freeze-dry-aiding agents such as amino acids.

The diagnostic imaging agent of the present invention may be administered orally or parenterally; for example, through a generally employed means such as intravenous

injection. Particularly, the nitroimidazole derivative (1) comprising a hydrogen atom as R^1 is water-soluble and tends to be directed to and accumulated in ischemic smooth muscle cells, or chemotherapeutic-agent-resistant or radiation-resistant cells in a tumor, and thus the derivative may be administered in the dosage form of injection. Meanwhile, the nitroimidazole derivative (1) comprising an alkanoyl group as R^1 may be administered orally as a prodrug in the dosage form of enteric-coated drug, since the alkanoyl group easily undergoes dealkanoylation in a living organism.

The derivative of the present invention is preferably administered about 2-3 hours before radiography or MRI.

The dose of the diagnostic imaging agent of the present invention is determined in consideration of various conditions such as the weight, age, and sex of a patient, and an imaging apparatus which is employed. When the diagnostic imaging agent is employed in MRI, the dose is preferably 0.1-10 g per person. When the agent is employed in PET, at least 0.01% of the agent is preferably replaced by the derivative comprising a radioisotope of fluorine. In PET, 1 ng-1 μg of the agent can be detected, and thus the dose of the agent may be reduced more.

Examples

The present invention will next be described in more detail by way of examples.

Reference Example 1

Synthesis of 1-[1-acetoxymethyl-2-

(hydroxy)ethoxy]methyl-2-nitroimidazole

2-Nitroimidazole was subjected to trimethylsilylation by use of hexamethyldisilazane in acetonitrile, and the resultant compound and 2-acetoxymethoxy-1,3-diacetoxypropane were subjected to condensation by use of stannic chloride serving as a catalyst. The resultant product was deprotected by use of methanolic ammonia, to thereby obtain 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole. The thus-obtained 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (0.5 g) was refluxed for two hours together with dibutyl tin oxide (0.6 g) in anhydrous toluene in the presence of molecular sieves having a pore size of 4 Å. The solvent was removed under reduced pressure, and anhydrous methylene chloride (16 mL) and anhydrous tetrahydrofuran (4 mL) were added to the residue. The resultant mixture was cooled to 0°C, and acetyl chloride (171 mg) was added to the mixture, and then the mixture was stirred for 30 minutes. To the resultant reaction mixture, a sodium phosphate buffer having a pH of 7.1 (10 mL) was added, and the resultant mixture was subjected to filtration. The resultant residue was subjected to extraction with chloroform (10 mL × 3), and the thus-obtained extract was mixed with the filtrate, and the mixture was separated, thereby obtaining an organic layer. The organic layer was dried over sodium sulfate, and then fractionated and purified through silica gel chromatography, to thereby yield the title compound, 1-[1-acetoxymethyl-2-(hydroxy)ethoxy]methyl-2-nitroimidazole (265 mg).

Example 1

Synthesis of 1-[2-(toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole (compound 1)

1-[1-Acetoxymethyl-2-(hydroxy)ethoxy]methyl-2-nitroimidazole (117 mg) was placed in a flask together with anhydrous pyridine, toluenesulfonyl chloride (252 mg) was added to the flask, and the resultant mixture was stirred at room temperature for five hours. The reaction mixture was subjected to extraction with ethyl acetate (30 mL), and the resultant extract was partitioned and washed with water (30 mL × 2). The resultant organic layer was dried over sodium sulfate, concentrated under reduced pressure, and purified through silica gel column chromatography, to thereby yield the title compound 1 (90.2 mg).

^{1}H−NMR (CD_3CN) : δ ppm

1.88 (s, 3H)、2.44 (s, 3H)、3.96～4.11 (m, 4H)、5.68、5.78 (AB pattern ; J＝1.0Hz、2H)、7.11 (d, J＝8.5Hz, 1H)、7.39 (d, J＝1.0Hz, 1H)、7.42 (d, J＝8.5Hz, 1H)、7.73 (d, J＝8.5Hz, 1H)

^{13}C−NMR (CD_3CN) : δ ppm

20.7、21.6、63.1、69.8、75.6、78.5、127.2、128.8、131.1、171.1

Mass spectrum : 413 (M^+)

Example 2

Synthesis of 1-[1-acetoxymethyl-2-fluoroethoxy]methyl-2-nitroimidazole (compound 2)

Acetonitrile (10 mL) was mixed with water (1 mL), and potassium fluoride (33.8 mg) and 18-crown-6 (80 mg) were added to the solution. The solution was dried under reduced pressure, compound 1 (89.2 mg) in anhydrous dimethylformamide (10 mL) was added to the above-dried solution, and the resultant mixture was heated at 110°C for eight hours. After ethyl acetate (20 mL) was added to the resultant reaction mixture, the mixture was washed with water (20 mL). The water layer was subjected to extraction with ethyl acetate (20 mL × 2), the resultant extract was mixed with the organic layer, and the resultant mixture was dried under reduced pressure. The dried product was purified through separable high performance chromatography, to thereby yield the title compound 2 (16.2 mg).

^{1}H−NMR (CD_3CN) : δ ppm

1.94 (s, 3H)、3.98～4.14 (m, 3H)、4.38～4.58 (m, 2H)、5.79、5.86 (AB pattern ; J＝1.2Hz、2H)、7.13 (d, J＝1.2Hz, 1H)、7.51 (d, J＝1.1Hz, 1H)

^{13}C−NMR (CD_3CN) : δ ppm

20.8、62.9、76.7、78.8、83.6、127.2、128.8、171.3

Mass spectrum : 261 (M^+)

Example 3

Synthesis of 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 3)

A 50 V/V% aqueous solution of ethanol (2 mL) containing

sodium hydroxide (0.05 N) was added to compound 2 of Example 2 (18 mg), and the mixture was stirred at 40°C for 1.5 minutes. The resultant reaction mixture was added to an ion exchange column to remove sodium cation. Thereafter, the resultant mixture was concentrated under reduced pressure, and then purified through separable high performance chromatography, to thereby yield the title compound 3 (10.3 mg).

$^{1}H-NMR$ (CD_3CN) : δ ppm

3.01 (br, 1H)、3.49～3.53 (m, 2H)、4.32～4.54 (m, 2H)、5.83、5.85 (AB pattern ; J＝10.8Hz、2H)、7.11 (d, J＝1.1Hz, 1H)、7.51 (d, J＝1.1Hz, 1H)

$^{13}C-NMR$ (CD_3CN) : δ ppm

61.1、79.1、79.9、84.1、127.0、128.8

Mass spectrum : 220.07 (M^{+1})

Example 4

Synthesis of 1-[2-fluoro(^{18}F)-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 4)

In a manner similar to those as described in Examples 1 and 2, 1-[2-(toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole was reacted with $K^{18}F$ (prepared by use of a cyclotron HW-12, 3.7 GBq) by use of cryptofix 2.2.2 serving as a phase transfer catalyst, to thereby yield the title compound 4 (150 MBq). Through high performance liquid chromatography, compound 4 was found

to have elution properties which are the same as those of 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 3) of Example 3. Therefore, compound 4 was found to be a compound in which a fluorine atom of compound 3 was replaced by ^{18}F.

Test Example 1

Imaging of an ischemic site of the heart was carried out by use of compound 4 of Example 4.

A male Donryu rat was anesthetized with pentobarbital, and the respiration of the rat was regulated by use of a respirator. The left chest of the rat was opened at the position between the seventh and eighth sterna, and the pericardium was incised for exposure of the heart. The left anterior descending artery stem of the coronary artery was ligated in order to induce ischemia. Separately, compound 4 was diluted with compound 3 so as to attain a radiation intensity of 150 MBq. The thus-diluted compound 4 was administered intravenously to the rat 15 minutes after completion of ligation. The heart was extirpated 40 minutes after administration of the compound 4, a frozen section of the heart was prepared, and the section was brought into contact with an imaging plate, to thereby obtain an autoradiogram thereof as shown in Fig. 1.

The autoradiogram revealed that the diagnostic imaging agent of the present invention exists at relatively high concentration at a muscle tissue site in the vicinity of the left ventricle, at which ischemia is usually generated by

such ligation, and thus the agent appropriately images an ischemic site.

Test Example 2

Compound 4 of Example 4 was intravenously injected to cancer-bearing mice (WHT/Ht albino mice) (3 mice per group). Systemic frozen sections of each mouse were prepared 10, 30, 60, 120, and 150 minutes after intravenous injection, and the radiation intensity of each organ was measured. Squamous-cell carcinoma and fibrosarcoma were employed as cancer sources. When the sections was prepared, blood was collected separately, and the ratio of radiation intensity in each organ to that in blood was obtained. The results are shown in Table 1.

Table 1

Organ	10 min.	30 min.	60 min.	120 min.	150 min.
Brain	1.168	0.815	0.308	0.116	0.126
Lung	2.151	1.347	0.596	0.353	0.331
Heart	1.721	0.929	0.378	0.216	0.186
Liver	10.300	5.018	1.692	0.730	0.800
Kidney	7.935	5.667	1.856	0.699	0.511
Muscle	1.576	0.742	0.314	0.139	0.159
Bone	1.157	0.617	0.244	0.253	0.218
Testis	0.861	0.852	0.466	0.412	0.139
Intestine	1.697	0.767	0.533	0.56	0.488
Blood	2.222	1.068	0.379	0.117	0.096
Fibrosarcoma	1.584	1.319	0.619	0.421	0.351
Fibrosarcoma/ blood	0.697	1.234	1.634	3.601	3.675
Squamous carcinoma	2.192	1.237	0.466	0.133	0.101
Squamous carcinoma/blood	0.893	1.012	1.384	2.904	2.357

Table 1 shows that the concentrations of compound (1) in the tumor are higher than in the blood or the other organs except those which are related with metabolism of this compound. Especially the ratio of the concentration in the tumor to that in the blood is in the range of 2-4. In addition, the concentrations of this compound in fibrosarcoma are higher than in squamous carcinoma, which has less hypoxic cells. Those teach us that the compound (1) of the present invention selectively distributes to the tumor, especially to the hypoxic sites of the tumor, and can be recognized due to the presence of a radioisotope.

In addition, an autoradiogram of a portion in the vicinity of the tumor was obtained 120 minutes after intravenous injection of the compound (1). The autoradiogram is shown in Fig. 2. The autoradiogram revealed that the compound (1) of the present invention is roughly spread over the entirety of the tumor relatively deeply. Also, it was found that the compound is located in the vicinity of necrotic portion of the tumor, and that the position at which the compound is located is identical with the position of chemotherapeutic-agent-resistant or radiation-resistant cancer cells. Therefore, the compound (1) of the present invention was found to be useful as a diagnostic imaging agent of cancer cells.

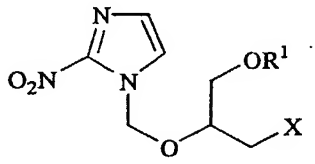
Industrial Applicability

The nitroimidazole derivative (1) of the present

invention enables imaging of the ischemic sites of circulatory organs, which are caused by circulatory organ diseases, or imaging of cancer cells such as agent-resistant or radiation-resistant hypoxic cancer cells, and thus the derivative can provide information about the position and the amount of ischemic sites or such cancer cells. Therefore, the derivative greatly contributes to selection of appropriate treatment of ischemia or cancer.

Claims

1. A nitroimidazole derivative represented by the following formula (1):



(1)

[wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group; and X represents a fluorine atom or an isotope thereof].

2. A nitroimidazole derivative according to claim 1, wherein X is ^{18}F.

3. A diagnostic imaging agent comprising, as an active ingredient, a nitroimidazole derivative as recited in claim 1 or 2.

4. A diagnostic imaging agent according to claim 3, which is used for imaging an ischemic site or cancer cell.

5. A diagnostic imaging agent according to claim 4, wherein the cancer cell is a chemotherapeutic-agent-resistant or radiation-resistant cancer cell.

6. Use of a nitroimidazole derivative according to claim 1 or 2 as a diagnostic imaging agent.

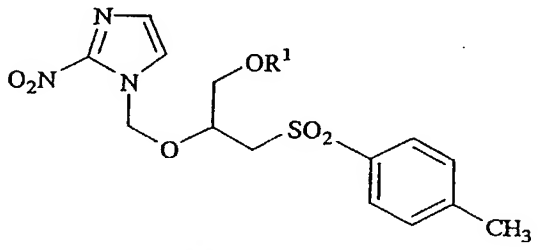
7. Use according to claim 6, wherein the diagnostic imaging agent is used for imaging an ischemic site or cancer cell.

8. A method of diagnostic imaging comprising administration of a nitroimidazole derivative as recited in

claim 1 or 2 for imaging.

9. A method of diagnostic imaging according to claim 8, wherein an ischemic site or cancer cell is imaged.

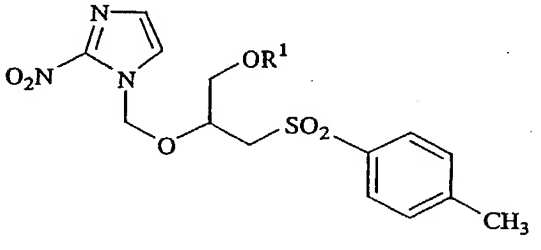
10. A nitroimidazole derivative represented by the following formula (2):



(2)

[wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group].

11. A method for producing a nitroimidazole derivative as recited in claim 1, comprising fluorination of a nitroimidazole derivative represented by the following formula (2):



(2)

[wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group].

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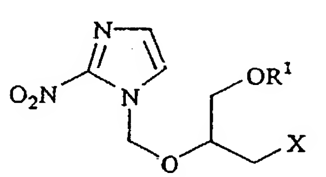
For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: NITROIMIDAZOLE DERIVATIVE AND DIAGNOSTIC IMAGING AGENT CONTAINING THE SAME



(1)

(57) **Abstract:** A nitroimidazole derivative represented by formula (1): [wherein R^1 represents a hydrogen atom or a C1-C4 alkanoyl group; and X represents a fluorine atom or an isotope thereof], and a diagnostic imaging agent containing the derivative as an active ingredient. The derivative enables imaging of the ischemic sites of a circulatory organ or imaging of cancer cells, and thus the derivative can provide information about the position and the amount of the ischemic sites or the cancer cells. Therefore, the derivative contributes to selection of appropriate treatment of ischemia or cancer.

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FIG. 1

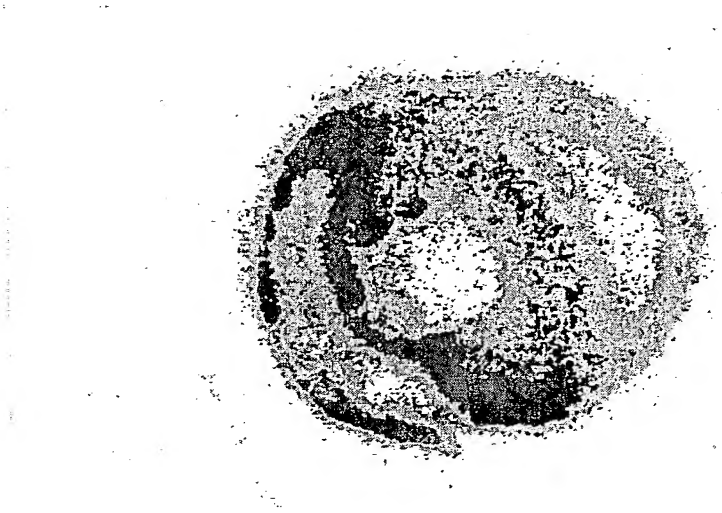
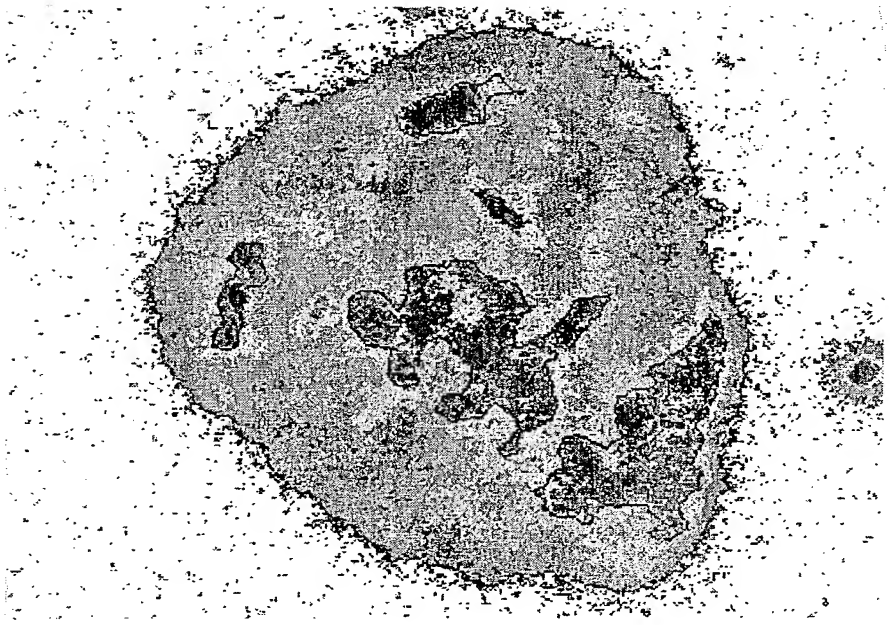


FIG. 2



Declaration and Power of Attorney For Patent Application

PC0018 USA
1/2

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

下記の氏名の発明者として、私は以下の通り宣言します。

私の住所、私書箱、国籍は下記の私の氏名の後に記載された通りです。

下記の名称の発明に関して請求範囲に記載され、特許出願している発明内容について、私が最初かつ唯一の発明者（下記の氏名が一つの場合）もしくは最初かつ共同発明者（下記の名称が複数の場合）であると信じています。

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上記発明の明細書は、

☐ 本書に添付されています。

☒ 2000年9月12日に提出され、米国出願番号または特許協定条約国際出願番号をPCT/JP00/06226とし、

（該当する場合）＿＿＿＿＿＿に訂正されました。

私は、特許請求範囲を含む上記訂正後の明細書を検討し、内容を理解していることをここに表明します。

私は、連邦規則法典第37編第1条56項に定義されるとおり、特許資格の有無について重要な情報を開示する義務があることを認めます。

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

NITROIMIDAZOLE DERIVATIVE AND DIAGNOSTIC IMAGING AGENT CONTAINING THE SAME

the specification of which

☐ is attached hereto.

☒ was filed on September 12, 2000 as ~~United States Application Number~~ or PCT International Application Number PCT/JP00/06226 and was amended on ＿＿＿＿＿＿ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

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私は、米国法典第35編119条（a）-（d）項又は365条（b）項に基づき下記の、米国以外の国の少なくとも一ヵ国を指定している特許協力条約365（a）項に基づく国際出願、又は外国での特許出願もしくは発明者証の出願についての外国優先権をここに主張するとともに、優先権を主張している、本出願の前に出願された特許または発明者証の外国出願を以下に、枠内をマークすることで、示しています。

I hereby claim foreign priority under Title 35, United States Code, Section 119 (a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s) 外国での先行出願			Priority Claimed 優先権主張	
11-259057	Japan	13/09/1999	x☐	☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Yes はい	No いいえ
11-260315	Japan	14/09/1999	x☐	☐
(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Yes はい	No いいえ

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I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Application No.) (出願番号)	(Filing Date) (出願日)

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I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of application.

(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)
(Application No.) (出願番号)	(Filing Date) (出願日)	(Status: Patented, Pending, Abandoned) (現況：特許許可済、係属中、放棄済)

私は、私自信の知識に基づいて本宣言書中で私が行なう表明が真実であり、かつ私の入手した情報と私の信じるところに基づく表明が全て真実であると信じていること、さらに故意になされた虚偽の表明及びそれと同等の行為は米国法典第18編第1001条に基づき、罰金または拘禁、もしくはその両方により処罰されること、そしてそのような故意による虚偽の声明を行なえば、出願した、又は既に許可された特許の有効性が失われることを認識し、よってここに上記のごとく宣誓を致します。

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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委任状：私は下記の発明者として、本出願に関する一切の手続きを米特許商標局に対して遂行する弁理士または代理人として、下記の者を指名いたします。
(弁護士、または代理人の指名及び登録番号を明記のこと)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

022850

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(Supply similar information and signature for third and subsequent joint inventors.)

Japanese Language Declaration
（日本語宣言書）

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第三の共同発明者の署名　辻谷典彦　日付　May 17,2002	Third joint Inventor's signature　Michihiko Tsujitani　Date　May 17,2002
住所　239-0814　日本国神奈川県横須賀市二葉２－１２－１	Residence　12-1, Futaba 2-chome, Yokosuka-shi, Kanagawa 239-0814 JAPAN
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（第六またはそれ以降の共同発明者に対しても同様な情報および署名を提供すること。）

(Supply similar information and signature for third and subsequent joint inventors.)